

Amendments to the Specification

Please replace the description of Figure 2, on page 10, with the following description:

Figure 2 shows the Primer oligonucleotides and PCR amplification of Ig α and Ig β cDNAs. (A) The sequences of the sense (S) (SEQ ID NO: 5 and SEQ ID NO: 7) and antisense (A) (SEQ ID NO: 6 and SEQ ID NO: 8) primers used to amplify cDNAs encoding Ig α and Ig β . These oligos add important restriction endonuclease cloning sites and 5' translation signals (GCCACC) to the receptor sequences. (B) PCR amplification products of the expected sizes in base pairs (bp). Molecular weight standards flank the PCR products.

Please replace the description of Figure 3, on pages 10 and 11, with the following description:

Figure 3 shows the sequence of PCR modified mouse Ig α cDNA (SEQ ID NO: 1) extending from the HindIII to EcoRI cloning sites used to construct Ig α expression plasmid p3.1NeoIg α . The Protein sequence (SEQ ID NO: 3) is shown above the DNA sequence. The main DNA and protein sequence listings in GenBank for Ig α have the accession numbers NM_007655 and NP_031681, respectively. The cDNA sequence obtained was inconsistent with this original Ig α sequence in a small region, but agrees with the data given by Sakaguchi et al. ("B lymphocyte lineage-restricted expression of mb-1, a gene with CD3-like structural properties" *EMBO J.* 7:3457-64 (1988)) encoding a protein with the sequence encoding six amino acids listed in bold.

Please replace the description of Figure 4, on pages 11, with the following description:

Figure 4 shows the sequence of PCR modified mouse Ig β cDNA (SEQ ID NO: 2) extending from the HindIII to EcoRI cloning sites used to construct Ig β expression plasmid p3.1NeoIg α . Protein sequence (SEQ ID NO: 4) is shown above the DNA

sequence. The main sequence listing in GenBank for Ig β has the accession number NM_008339.

Please replace the first paragraph on page 71 with the following paragraph:

Engineering the constitutive expression of Ig α and/or Ig β : The cDNAs encoding the two receptor sequences Ig α and Ig β were PCR amplified from a mouse spleen cDNA library (Clontech). Restriction endonuclease cloning sites were added as part of the oligonucleotide primers used in the PCR amplification as shown in **Figure 2A** and the appropriate-sized PCR products were obtained (**Figure 2B**). The confirmed sequences of the PCR-amplified receptor for Ig α and Ig β are shown in **Figures 3 and 4**, respectively. The PCR product containing Ig α was digested with *Hind*III and *Eco*RI and cloned into the corresponding replacement region of the eukaryotic expression vector pcDNA3.1 (Neo) (Invitrogen, Inc.). The PCR product containing the Ig β sequence was digested with *Hind*III and *Xho*I and cloned into the corresponding replacement regions of the eukaryotic expression vector pcDNA3.1/Zeo (Invitrogen, Inc.). The structure of these two related pcDNA3.1 expression vectors are shown in **Figures 5 and 6**, respectively. The two vectors differ only in carrying resistance markers for Neomycin G418 and ~~Zeo~~in ZEOCINTM, respectively. The resulting plasmids are termed p3.1NeoIg α and p3.1ZeoIg β , respectively. Both pcDNA3.1 vectors express cloned sequences under the control of the strong constitutive CMV promoter and BGH terminator. Recombinant plasmid DNA was purified over an endotoxin free purification kit (Qiagen, Inc.) in preparation for transfection.

Please replace the paragraph bridging pages 71 and 72 with the following paragraph:

Optimizing transfection and selection: A constitutive β -galactosidase (β -gal) reporter plasmid (pcDNA3.1/lacZ, Invitrogen) was utilized to optimize and quantify lipofection techniques on HGS1 hybridoma and Sp2/0 myeloma cell lines. Transfection was performed by mixing 6-8 μ l of LipofectAMINE reagent (Gibco BRL) with 1-2 μ g of

plasmid DNA for 5 hr at 37°C in 1.0 ml of Opti-MEM I (GibcoBRL) reduced serum medium. Lipofection frequencies that occurred were relatively low, averaging approximately 30 transfectants per 500,000 cells, but were higher than previously reported for myeloma cells (Oi et al., 1983; Sun et al., 1991). The frequency of co-transfection of two DNAs was determined to average about 6-10 cells per 500,000. There was little difference between the frequency of transfecting or expressing linear or supercoiled plasmid DNA in several transfections, therefore, supercoiled DNA was used for subsequent experiments. Neomycin (Neo) (G418, Gibco BRL) and Zeocin (Invitrogen) kill curves were established on the same cells with 100% killing of control cells occurring over 7 days on 750 µg/ml G418 and 750 mg/ml ~~Zeocin~~ ZEOCIN™. After this initial period of selection the G418 concentration remains the same, but the ~~Zeocin~~ ZEOCIN™ concentration is reduced to 450 µg/ml. Cells are grown under continuous selection.

Please replace the paragraph bridging pages 72 and 73 with the following paragraph:

Transfection and expression of Ig receptor genes: Receptor protein levels were assayed on Western blots of crude extracts resolved by SDS-PAGE. Rabbit polyclonal antibodies to the two receptors were provided by Dr. Linda Matsuuchi (Univ. Vancouver). Strong receptor expression is seen in the 30-40 kDa range for the spleen cell control (SC) as shown in **Figure 7**, while the higher molecular weight bands appear to be background. Using a double-drug selection for Neomycin and ~~Zeocin~~ ZEOCIN™ isolated several independent and stably co-transfected cell lines (HGS1αβ1-HGS1αβ16) containing the two constructs p3.1NeoIgα and p3.1ZeoIgβ were isolated. Several of these cell lines were examined for Igα and Igβ expression on western blots. Two of five lines examined in one experiment, HGS1-Igαβ2 and HGS1-Igαβ5 (**Figure 7**) produced measurable levels of Igα protein. This experiment also revealed that all cell lines examined produced significant amounts of Igβ with or without transfection with the pNeo3.1Igα and p3.1ZeoIgβ constructs. This background expression of Igβ was observed in myeloma line Sp2/0, the hybridoma line HGS1 (derived from a fusion

between Sp2/0 and a mouse B-cell), all lines derived from HGS1, and other hybridoma lines derived from Sp2/0.

Please replace the first full paragraph on page 73 with the following paragraph:

Increased surface presentation of antibody in transfected hybridoma lines: The lines expressing high levels of Ig α from p3.1NeoIg α were examined for surface presentation of antibody in **Figure 8**. FITC-labeled sheep polyclonal anti-mouse antibody (Sigma) was used to measure the base level of mouse antibody on the surface of control cells, HGS1a. A low frequency of control cells present antibody with the typical result from several experiments being shown in **Figure 8A-B**. Remarkably, four of the six HGS1 $\alpha\beta$ cell lines transfected with both receptor plasmids ($\alpha\beta 6$, $\alpha\beta 7$, $\alpha\beta 9$, $\alpha\beta 10$) present large amounts of antibody on the surface of 100% of their cells as shown in **Figure 8C, D, F, and G**, respectively. A few cells in each field are out of focus, but a through focus examination of the field of cells reveals that 99% of the cells in each of the four populations present detectable levels of surface antibody. Clearly, examination of these cell populations reveals a significant increase in both the frequency of the cells that present antibody relative to the control cells and increases in the level of expression. Two of the G418 and Zeocin ZEOCINTM resistant transfected cell lines ($\alpha\beta 8$, $\alpha\beta 11$) showed no significant surface presentation of antibody (**Figure 8E and 8H**). Surprisingly, they presented less surface antibody, even than control HGS1 cells, with none of the 100-plus cells examined showing detectable surface expression.

Please replace the first full paragraph on page 75 with the following paragraph:

Myelomas The same genetic alteration utilized above on hybridoma cells is performed on a standard myeloma fusion partner Sp2/0. Sp2/0 is a myeloma cell line obtained from mice, *Mus musculus* (BALB/c). Sp2/0 is one of the founding myeloma cell lines used to make hybridoma fusions (Fraser and Venter, 1980; Greene et al., 1980; Hurwitz et al., 1980). First, Sp2/0 cells were co-transfected with the p3.1NeoIg α and p3.1ZeoIg β constructs and selected for G418 and Zeocin ZEOCINTM resistance, to

produce new cell lines Sp2 $\alpha\beta$ 1, Sp2 $\alpha\beta$ 2, etc. These Sp2 $\alpha\beta$ lines were characterized for Ig α and Ig β receptor expression on western blots as shown in **Figure 12**. Lines Sp2 $\alpha\beta$ 1 and Sp2 $\alpha\beta$ 2, show strong Ig α expression, and demonstrate that there is no post-transcriptional barrier to increasing receptor expression in myeloma cells. It appears that Ig β is already expressed at measurable levels in the control Sp2/0 control cells. These myeloma cell lines are ready to be fused with B cells in order to make hybridomas. Myeloma cells can be fused to a B cell or other antibody producing cell by methods standard in the art.

Please replace the fourth full paragraph on page 83 with the following paragraph:

Signals. (2000). Companies load up on magic bullets, Signals Magazine **October**, 1-9
(<http://www.recap.com/signalsma>).